

SUPPLEMENTARY APPENDIX

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Table S1. Staining antibodies used for analysis of cellular composition of CART products with flow cytometry.

Antibodies	Vendor	Staining round
CD19-BUV496	BD	1
CAR T detection reagent	Miltenyi	2
CD16-BUV395	BD	3
CD33 BUV563	BD	3
CD314 BUV615	BD	3
CD27 BUV661	BD	3
CD8 BUV737	BD	3
CD45 BUV805	BD	3
CD141 BV421	Biologend	3
IgD Pacific Blue	Biologend	3
CD39 BV480	BD	3
ICOS BV510	BD	3
CD45RO BV570	Biologend	3
CD11c BV605	Biologend	3
CD279 BV650	BD	3
CD56 BV711	Biologend	3
TCRab BV750	BD	3
CD45RA BV785	BD	3
CD11b BB515	BD	3
CD3 Spark Blue550	Biologend	3
CD38 PerCP	Biologend	3
CD94 BB700	BD	3
TCRgd PerCP-efluor710	Thermo Fisher	3
CD1c PE-Dazzle 594	Biologend	3
CD95 PE-Fire640	Biologend	3
ITGB7 PE-Cy5	BD	3
CD25 PE-Fire700	Biologend	3
FcεR1α PE-Cy7	Invitrogen	3
CD4 PE-Fire810	Biologend	3
CD197 APC	BD	3
CD123 Alexa Fluor 647	Biologend	3
CD14 Spark-NIR 685	Biologend	3
CD127 APC R700	BD	3
CD34 APC-Cy7	Biologend	3
HLA-DR APC-Fire810	Biologend	3
Zombie NIR (Fixable viability kit)	Biologend	3
Brilliant Stain buffer plus	BD	3

Table S2. Characteristics and cellular composition of HD-CAR-1 CART products.

UPN	Total cells	Viability	CD3 ⁺ CAR ⁺ T cells	CD4 ⁺ T cells	CD8 ⁺ T cells	CD4 ⁺ CD8 ⁺ T cells	CD4 ⁻ CD8 ⁻ T cells	CD45RA ⁻ CD197 ⁺ T cells	CD45RA ⁺ CD197 ⁺ T cells	CD45RA ⁻ CD197 ⁻ T cells	CD45RA ⁺ CD197 ⁻ T cells	Manufacturing (days)
1	298 x 10 ⁶	94,9%	41,7%	63,3%	25,6%	0,8%	10,3%	23,6%	22,5%	33,1%	20,8%	10
2	107 x 10 ⁶	86,2%	48,5%	39,8%	52,3%	5,2%	2,7%	27,8%	44,5%	12,7%	15,0%	10
3	599 x 10 ⁶	91,7%	54,5%	60,4%	32,4%	1,9%	5,3%	34,4%	24,2%	25,5%	15,9%	10
4	1084 x10 ⁶	95,1%	67,7%	56,9%	39,8%	2,0%	1,4%	25,0%	19,7%	36,4%	18,9%	13
5	874 x10 ⁶	95,5%	62,3%	77,0%	16,7%	5,3%	1,0%	19,9%	16,2%	44,0%	19,9%	13
6	1432 x10 ⁶	95,6%	52,3%	50,7%	41,8%	1,7%	5,8%	17,3%	19,9%	38,6%	24,2%	13
7	840 x10 ⁶	95,8%	59,9%	62,3%	30,1%	0,5%	7,2%	19,7%	21,5%	39,6%	19,1%	13
8	922 x10 ⁶	95,5%	43,8%	71,7%	25,7%	0,5%	2,1%	19,8%	12,5%	54,5%	13,1%	13
9	1078 x10 ⁶	95,2%	50,5%	60,6%	37,0%	0,6%	1,8%	12,5%	5,5%	71,5%	10,5%	13

HD-CAR-1 CART products were analyzed with flow cytometry. Cells were stained with following antibodies: CD3 (clone SK7), CD4 (clone SK3), CD8 (clone SK1), CD45 (clone 2D1), CD45RA (clone HI100) and CD197 (clone G043H7). For detection of dead cells, LIVE/DEAD fixable NEAR-IR (Thermo Fisher Scientific), and for detection of CD19-directed HD-CAR-1 CAR positive cells, the biotinylated CD19 CAR Detection Reagent and the anti-biotin antibody (both Miltenyi Biotec) were applied. Samples were measured on BD FACSCanto Flow Cytometry Cell Analyzer (BD Biosciences). Data analysis was conducted with DIVA (BD Biosciences, version 8.0). Cells were counted with LunaFL Cell Counter (Logos Biosystems).